ARCHITECT

SYSTEM

en

HBsAg Qualitative

REF 4P53

34-6722/R01

B4P530

HBsAg Qualitative

Customer Service: Contact your local representative or find country specific contact information on www.abbottdiagnostics.com

Caution: United States Federal Law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory; and use is restricted to, by, or on the order of a physician.

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Key to symbols used

2°C–8°C	Store at 2-8°C	IVD	*In Vitro* Diagnostic Medical Device
SN	Serial number	CONTROL NO.	Control Number
REF	List number	REACTION VESSELS	Reaction Vessels
LOT	Lot number	REAGENT LOT	Reagent Lot
	Expiration Date	REPLACEMENT CAPS	Replacement Caps
	Consult instructions for use	SAMPLE CUPS	Sample Cups
	Manufacturer	SEPTUM	Septum
	Caution	WARNING: SENSITIZER	WARNING: May cause an allergic reaction

See **REAGENTS** section for a full explanation of symbols used in reagent component naming.

NAME

ARCHITECT HBsAg Qualitative

INTENDED USE

The ARCHITECT HBsAg Qualitative assay is a chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of hepatitis B surface antigen (HBsAg) in human adult and pediatric serum and plasma and neonate serum. The assay may also be used to screen for HBV infection in pregnant women to identify neonates who are at risk for acquiring hepatitis B during the perinatal period. Assay results, in conjunction with other laboratory results and clinical information, may be used to provide presumptive evidence of infection with the hepatitis B virus (HBV) (state of infection or associated disease not determined) in persons with signs and symptoms of hepatitis and in persons at risk for hepatitis B infection.

Not intended for use in screening blood, plasma, or tissue donors.

SUMMARY AND EXPLANATION OF TEST

The causative agent of serum hepatitis is hepatitis B virus (HBV) which is an enveloped DNA virus. During infection, HBV produces an excess of hepatitis B surface antigen (HBsAg), also known as Australia antigen, which can be detected in the blood of infected individuals. It is responsible for binding the virus to the liver cell and is the target structure of neutralizing antibodies.[1,2] HBsAg is the first serological marker after infection with HBV, appearing one to ten weeks after exposure and two to eight weeks before the onset of clinical symptoms.[3,4] HBsAg persists during this acute phase and clears late in the convalescence period. Failure to clear HBsAg within six months indicates a chronic HBsAg carrier state.

HBsAg assays are used to identify persons infected with HBV and to monitor the status of infected individuals in combination with other hepatitis B serological markers.[5] In most countries, testing for HBsAg is part of the antenatal screening program to identify HBV infected mothers and to prevent perinatal HBV infection by subsequent immunization.[6]

Specimens nonreactive by ARCHITECT HBsAg Qualitative are considered negative for HBsAg. A reactive specimen must be retested in duplicate by ARCHITECT HBsAg Qualitative to determine whether it is repeatedly reactive. Specimens found to be repeatedly reactive by the ARCHITECT HBsAg Qualitative assay should be confirmed using the ARCHITECT HBsAg Qualitative Confirmatory (4P54) assay, a neutralization procedure utilizing human anti-HBs. If the specimen is neutralized, the specimen is considered confirmed positive for HBsAg. It is recommended that confirmatory testing be performed before disclosing HBsAg status.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT HBsAg Qualitative assay is a one-step immunoassay for the qualitative detection of HBsAg in human serum and plasma using CMIA technology, with flexible assay protocols, referred to as Chemiflex. (Note: Ancillary Wash Buffer is added in a second incubation step so the assay files perform a two-step assay.)

In the ARCHITECT HBsAg Qualitative assay, sample, anti-HBs coated paramagnetic microparticles, and anti-HBs acridinium-labeled conjugate are combined to create a reaction mixture. HBsAg present in the sample binds to the anti-HBs coated microparticles and to the anti-HBs acridinium-labeled conjugate. After washing, ancillary wash buffer is added to the reaction mixture. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of HBsAg in the sample and the RLUs detected by the ARCHITECT *i* System optics.

The presence or absence of HBsAg in the sample is determined by comparing the chemiluminescent signal in the reaction to the cutoff signal determined from an active calibration. If the chemiluminescent signal in the specimen is greater than or equal to the cutoff signal, the sample is considered reactive for HBsAg.

For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

REAGENTS

Reagent Kit, 100 Tests/500 Tests

NOTE: Some kit sizes are not for use on all ARCHITECT *i* Systems. Please contact your local distributor.

ARCHITECT HBsAg Qualitative Reagent Kit (4P53)

- **MICROPARTICLES** 1 bottle (6.6 mL per 100-test bottle/27.0 mL per 500-test bottle) anti-HBs (mouse, monoclonal, IgM, IgG) coated microparticles in MES buffer with protein (bovine serum albumin) stabilizer. Minimum concentration: 0.08% solids. Preservatives: ProClin 300 and ProClin 950.
- **CONJUGATE** 1 bottle (5.9 mL per 100-test bottle/ 26.3 mL per 500-test bottle) anti-HBs (mouse, monoclonal, IgG) and anti-HBs (goat, IgG) acridinium-labeled conjugate in phosphate buffer with human plasma and protein (bovine serum albumin, fetal bovine serum, goat IgG, mouse IgG) stabilizers. Minimum concentration: 0.35 µg/mL. Preservatives: ProClin 300 and ProClin 950.
- **ANCILLARY WASH BUFFER** 1 bottle (5.9 mL per 100-test bottle/ 26.3 mL per 500-test bottle) ancillary wash buffer containing MES buffer. Preservatives: ProClin 300 and ProClin 950.

Other Reagents

ARCHITECT *i* Pre-Trigger Solution

- **PRE-TRIGGER SOLUTION** Pre-trigger solution containing 1.32% (w/v) hydrogen peroxide.

ARCHITECT *i* Trigger Solution

- **TRIGGER SOLUTION** Trigger solution containing 0.35 N sodium hydroxide.

ARCHITECT *i* Wash Buffer

- **WASH BUFFER** Wash buffer containing phosphate buffered saline solution. Preservatives: antimicrobial agents.

WARNINGS AND PRECAUTIONS

- **IVD**
- For *In Vitro* Diagnostic Use.
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

- **CAUTION:** This product contains human sourced and/or potentially infectious components. Refer to the **REAGENTS** section of this package insert. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens.[7] Biosafety Level 2[8] or other appropriate biosafety practices[9,10] should be used for materials that contain or are suspected of containing infectious agents.
- The Conjugate contains human plasma that is nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, and anti-HCV.
- The following warnings and precautions apply to these components:
  - Microparticles
  - Conjugate
  - Ancillary Wash Buffer

	WARNING:	Contains methylisothiazolones
	H317	May cause an allergic skin reaction.
	Prevention	
	P261	Avoid breathing mist / vapours / spray.
	P272	Contaminated work clothing should not be allowed out of the workplace.
	P280	Wear protective gloves / protective clothing / eye protection.
	Response	
	P302+P352	IF ON SKIN: Wash with plenty of water.
	P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
	P363	Wash contaminated clothing before reuse.

This material and its container must be disposed of in a safe way.

- For a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

42

Handling Precautions

- Do not use reagents kits beyond the expiration date.
- **Do not pool reagents within a kit or between reagent kits.**
- Before loading the ARCHITECT HBsAg Qualitative Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. For microparticle mixing instructions, refer to the **PROCEDURE, Assay Procedure** section of this package insert.
- **Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.**
  - **To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.**
  - Once a septum has been placed on the reagent bottle, **do not invert the bottle** as this will result in reagent leakage and may compromise assay results.
  - Over time, residual liquids may dry on the septum surface. These are typically dried salts, and have no effect on assay efficacy.
- For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Storage Instructions

- The ARCHITECT HBsAg Qualitative Reagent Kit must be stored at 2-8°C in an upright position and may be used immediately after removal from 2-8°C storage.
- When stored and handled as directed, the reagents are stable until the expiration date.
- The ARCHITECT HBsAg Qualitative Reagent Kit may be stored on board the ARCHITECT *i* System for a maximum of 30 days. After 30 days, the reagent kit must be discarded. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.
- Reagents may be stored on or off the ARCHITECT *i* System. If reagents are removed from the system, store them at 2-8°C (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright. **If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded.** For information on unloading reagents, refer to the ARCHITECT System Operations Manual, Section 5.

Indications of Reagent Deterioration

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results are invalid and samples must be retested. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

- The ARCHITECT HBsAg Qualitative assay is designed for use on the ARCHITECT *i* System.
- ARCHITECT System software version 7.00 or higher must be installed on the ARCHITECT *i* System.
- The ARCHITECT HBsAg Qualitative assay file (assay number 636) must be installed on the ARCHITECT *i* System before performing the assay.
- For detailed information on assay file installation and viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.
- For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.
- For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

- The specimen collection tubes listed below were verified for use with the ARCHITECT HBsAg Qualitative assay. Other specimen collection tubes have not been tested with this assay.
  - Human serum (including serum collected in serum separator tubes)
  - Human plasma collected in lithium heparin (including separator tubes), dipotassium EDTA, tripotassium EDTA, or sodium heparin
- Performance has not been established for the use of cadaveric specimens or the use of body fluids other than human serum and plasma.
- Liquid anticoagulants may have a dilution effect resulting in lower S/CO values for individual patient specimens.
- The ARCHITECT *i* System does not provide the capability to verify specimen type. It is the responsibility of the operator to verify that the correct specimen types are used in the ARCHITECT HBsAg Qualitative assay.

Specimen Conditions

- Do not use specimens with the following conditions:
  - heat-inactivated
  - pooled
  - grossly hemolyzed
  - obvious microbial contamination
- **For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter.** Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.
- As specimens from heparinized patients may be partially coagulated and erroneous results could occur due to the presence of fibrin, draw the specimen prior to heparin therapy.
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- For optimal results, inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for serum and plasma collection tubes. Gravity separation is not sufficient for specimen preparation.
- Prepare frozen specimens as follows:
  - Frozen specimens must be completely thawed before mixing.
  - Mix thawed specimens thoroughly by inverting 10 times or by low speed vortexing. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous. **If samples are not mixed thoroughly, inconsistent results may be obtained.**
  - Centrifuge mixed specimens as described below.
- To ensure consistency in results, specimens must be transferred to a centrifuge tube and centrifuged at ≥ 10,000 RCF (Relative Centrifugal Force) for 10 minutes before testing if
  - they contain fibrin, red blood cells, or other particulate matter or
  - they were frozen and thawed.
- Centrifuged specimens with a lipid layer on the top must be transferred to a sample cup or secondary tube. Care must be taken to transfer only the clarified specimen without the lipemic material.
- Transfer clarified specimen to a sample cup or secondary tube for testing.

Storage

- Specimens may be stored on or off the clot, red blood cells, or separator gel for
  - up to 24 hours at room temperature (15-30°C) or.
  - up to 6 days at 2-8°C.
- If testing will be delayed more than 6 days, remove serum or plasma from the clot, red blood cells, or separator gel and store at -20°C or colder.
- Avoid more than 3 freeze/thaw cycles.
  - Lithium heparin tube type may demonstrate higher S/CO values for low positive specimens after freeze/thaw.

Shipping

- Before shipping specimens, it is recommended that specimens be removed from the clot, red blood cells, or separator gel.
- When shipping specimens, package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.
- Specimens may be shipped ambient, at 2-8°C (wet ice), or frozen (dry ice). Do not exceed the storage time limitations listed above.

PROCEDURE

Materials Provided

- 4P53 ARCHITECT HBsAg Qualitative Reagent Kit

Materials Required but not Provided

- ARCHITECT *i* System
- ARCHITECT HBsAg Qualitative Assay file, obtained from the
  - ARCHITECT *i* System e-Assay CD-ROM; obtain from www.abbottdiagnostics.com or
  - ARCHITECT *i* System Assay CD-ROM
- 4P53-01 ARCHITECT HBsAg Qualitative Calibrators
- 4P53-10 ARCHITECT HBsAg Qualitative Controls (or other control material)
- ARCHITECT *i* **PRE-TRIGGER SOLUTION**
- ARCHITECT *i* **TRIGGER SOLUTION**
- ARCHITECT *i* **WASH BUFFER**
- ARCHITECT *i* **REACTION VESSELS**
- ARCHITECT *i* **SAMPLE CUPS**
- ARCHITECT *i* **SEPTUM**
- ARCHITECT *i* **REPLACEMENT CAPS**
- Pipettes or pipette tips (optional) to deliver the specified volumes.

For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.

Assay Procedure

- Before loading the ARCHITECT HBsAg Qualitative Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. After the first time the microparticles have been loaded, no further mixing is required.
  - **Invert the microparticle bottle 30 times.**
  - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
  - **If the microparticles do not resuspend, DO NOT USE. Contact your Abbott representative.**
  - Once the microparticles have been resuspended, place a septum on the bottle. For instructions about placing septums on bottles, refer to the **Handling Precautions** section of this package insert.
- Load the ARCHITECT HBsAg Qualitative Reagent Kit on the ARCHITECT *i* System.
  - Verify that all necessary reagents are present.
  - Ensure that septums are present on all reagent bottles.
- Order calibration, if necessary.
  - For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.
- Order tests.
  - For information on ordering patient specimens and controls and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
- The minimum sample cup volume is calculated by the system and is printed on the Orderlist report. No more than 10 replicates may be sampled from the same sample cup. To minimize the effects of evaporation, verify adequate sample cup volume is present before running the test.
  - Priority: 125 µL for the first HBsAg Qualitative test plus 75 µL for each additional HBsAg Qualitative test from the same sample cup.
  - ≤ 3 hours on-board: 150 µL for the first HBsAg Qualitative test plus 75 µL for each additional HBsAg Qualitative test from the same sample cup.
  - > 3 hours on-board: replace with a fresh sample (patient specimens, controls, and calibrators).
  - If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
- Prepare calibrators and controls.
  - Mix the ARCHITECT HBsAg Qualitative Calibrators and Controls by gentle inversion before use.
  - To obtain the recommended volume requirements for the ARCHITECT HBsAg Qualitative Calibrators and Controls, hold the bottles **vertically**, and dispense 11 drops of each calibrator and 6 drops of each control into each respective sample cup.
  - If commercially available control material is used, follow the manufacturer's instructions for preparation.
- Load samples
  - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN.
- For additional information on principles of operation, refer to the ARCHITECT System Operations Manual, Section 3.
- For optimal performance, it is important to perform routine maintenance as described in the ARCHITECT System Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

Specimen Dilution Procedures

Specimens cannot be diluted for the ARCHITECT HBsAg Qualitative assay.

Calibration

- To perform an ARCHITECT HBsAg Qualitative calibration, test calibrators 1 and 2 in replicates of 3. The calibrators should be priority loaded.
- A single sample of each control level must be tested to evaluate the assay calibration.
  - Order controls as described in the **Assay Procedure** section.
  - Ensure that assay control values are within the ranges specified in the control package insert.
- Once an ARCHITECT HBsAg Qualitative calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - A reagent kit with a new lot number is used.
  - Controls are out of range.
- For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

QUALITY CONTROL PROCEDURES

The recommended control requirement for the ARCHITECT HBsAg Qualitative assay is that a single sample of each control be tested once every 24 hours each day of use. If your laboratory quality control procedures require more frequent use of controls to verify test results, follow those procedures. Additional controls may be tested in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control policy.

Control values must be within the ranges specified in the control package insert. If a control result is out of its specified range, any test results generated since the last acceptable control results must be evaluated to determine if test results may have been adversely affected.[11,12] Adversely affected test results are invalid, and these samples must be retested. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B. The ARCHITECT HBsAg Qualitative assay belongs to method group 5, except functional sensitivity.

RESULTS

Calculations

- The ARCHITECT *i* System calculates the result for the ARCHITECT HBsAg Qualitative assay using the ratio of the sample RLU to the cutoff RLU (S/CO) for each specimen and control.
  - Cutoff RLU = (0.0575 x Calibrator 1 Mean RLU) + (0.8 x Calibrator 2 Mean RLU)
  - S/CO = Sample RLU/Cutoff RLU

Interpretation of Results

ARCHITECT HBsAg Qualitative Initial Result

Initial Result (S/CO)	Instrument Interpretation	Retest Procedure
< 1.00	NONREACTIVE	No retest required.
≥ 1.00	REACTIVE	Retest in duplicate.

- A specimen with an S/CO of less than 1.00 is nonreactive; the specimen is considered negative for HBsAg.
- Initially reactive specimens require retesting. Specimens that contain particulate matter should be recentrifuged according to directions in the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section in this package insert.

ARCHITECT HBsAg Qualitative Retest Results

Instrument Interpretation	Specimen Classification
Both results nonreactive (both S/CO values < 1.00)	Specimen considered negative for HBsAg.
One or both results reactive (one or both S/CO values ≥ 1.00)	Specimen considered repeatedly reactive; confirm using the ARCHITECT HBsAg Qualitative Confirmatory assay.

- Confirm repeatedly reactive specimens using the ARCHITECT HBsAg Qualitative Confirmatory assay before disclosing HBsAg status to the patient.

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the ARCHITECT System Operations Manual, Section 5.

LIMITATIONS OF THE PROCEDURE

- The effectiveness of the ARCHITECT HBsAg Qualitative assay for use in screening blood, plasma, or tissue donors has not been established.
- Assay performance characteristics have not been established when the ARCHITECT HBsAg Qualitative assay is used in conjunction with other manufacturers' assays for specific HBV markers. Users are responsible for establishing their own performance characteristics.
- Current methods for the detection of hepatitis B surface antigen may not detect all potentially infected individuals. A nonreactive test result does not exclude the possibility of exposure to or infection with hepatitis B virus. A nonreactive test result in individuals with prior exposure to hepatitis B may be due to antigen levels below the detection limit of this assay or lack of antigen reactivity to the antibodies in this assay.
- If the ARCHITECT HBsAg Qualitative results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- For diagnostic purposes, results should be used in conjunction with patient history and other hepatitis markers for diagnosis of acute and chronic infection.
- Results obtained with the ARCHITECT HBsAg Qualitative assay may not be used interchangeably with values obtained with different manufacturers' assay methods.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[13] Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous results may be observed. Additional information may be required for diagnosis.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA).[14,15] Specimens containing HAMA may produce anomalous values when tested with assay kits such as ARCHITECT HBsAg Qualitative that employ mouse monoclonal antibodies.[14]
- A reactive HBsAg result does not exclude co-infection by another hepatitis virus.
- Refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section of this package insert for specimen limitations.

EXPECTED RESULTS

Data in the **EXPECTED RESULTS** section were generated using the ARCHITECT *i* 2000_{SR} and *i* 2000 Systems.

Due to geographic locations or demographics, assay results obtained in individual laboratories may vary from the data presented.

Increased Risk Population

Of the 2800 specimens tested in the ARCHITECT HBsAg Qualitative clinical study, 1279 specimens were from individuals with increased risk of HBV infection. All 1279 individuals were at risk for HBV infection due to lifestyle, behavior, occupation, or a known exposure event but were asymptomatic and reported no current signs or symptoms of hepatitis. Testing of these specimens was performed at three clinical sites located in Hershey, PA; Fort Lauderdale, FL; and Aurora, CO.

The increased risk population (n=1279) consisted of the following race/ethnic groups:

- 598 (46.76%) Caucasian
- 470 (36.75%) African American
- 159 (12.43%) Hispanic
- 25 (1.95%) Asian
- 2 (0.16%) American Indian/Alaska Native
- 25 (1.95%) Other

The percentage of specimens collected at each location and the percentage of reactive results from each location are presented in the following table.

Specimen Collection Site/ Vendor Location	Percent of Specimens Collected at Each Location	Percent of Reactive Results from Each Location
Site 1 Galveston, TX	26.66 (341/1279)	2.05 (7/341)
Site 2 Dallas, TX	9.46 (121/1279)	4.13 (5/121)
Site 3 Miami, FL	8.68 (111/1279)	18.92 (21/111)
Site 4 St. Petersburg, FL	33.39 (427/1279)	0.70 (3/427)
Site 5 Chicago, IL	5.47 (70/1279)	8.57 (6/70)
Site 6 Denver, CO	2.42 (31/1279)	3.23 (1/31)
Specimen Vendor Location		
High Point, NC	2.58 (33/1279)	3.03 (1/33)
Colton, CA	2.66 (34/1279)	0.00 (0/34)
Plymouth, MA	8.68 (111/1279)	0.00 (0/111)
Total	**100.00 (1279/1279)**	**3.44 (44/1279)**

Of the 1279 specimens, 607 (47.46%) were female and 672 (52.54%) were male. The age was not reported for two subjects. Of the remaining 1277 specimens, the mean age was 39 years (age range: 17 to 82 years).

The distribution of ARCHITECT HBsAg Qualitative reactive and nonreactive results among the increased risk population by age and gender (n=1279) is summarized in the following table.

Age Range (Years)	Gender	ARCHITECT HBsAg Qualitative Result		Total
		Number of Reactive (%)	Number of Nonreactive %)	
10 to 19	Female	1 (7.69)	12 (92.31)	13
	Male	2 (18.18)	9 (81.82)	11
20 to 29	Female	2 (1.12)	176 (98.88)	178
	Male	2 (1.36)	145 (98.64)	147
30 to 39	Female	3 (2.65)	110 (97.35)	113
	Male	8 (4.71)	162 (95.29)	170
40 to 49	Female	1 (0.63)	159 (99.38)	160
	Male	4 (1.90)	206 (98.10)	210
50 to 59	Female	5 (5.05)	94 (94.95)	99
	Male	12 (11.21)	95 (88.79)	107
60 to 69	Female	4 (11.11)	32 (88.89)	36
	Male	0 (0.00)	17 (100.00)	17
70 to 79	Female	0 (0.00)	5 (100.00)	5
	Male	0 (0.00)	9 (100.00)	9
80 to 89	Female	0 (0.00)	2 (100.00)	2
	Male	0 (0.00)	0 (0.00)	0
Unknown	Female	0 (0.00)	1 (100.00)	1
	Male	0 (0.00)	1 (100.00)	1
Total		**44 (3.44)**	**1235 (96.56)**	**1279**

SPECIFIC PERFORMANCE CHARACTERISTICS

All performance studies were conducted using the ARCHITECT *i* 2000/*i* 2000$_{SR}$ Systems.

Assay results obtained in individual laboratories may vary from data presented.

Precision

The ARCHITECT HBsAg Qualitative assay is designed to have a Within-Laboratory (Total) imprecision %CV of ≤ 10% for the positive control and specimens at 1.20 S/CO (low positive panel) and 3.5 S/CO (moderate positive panel) and a Total Standard Deviation (SD) of ≤ 0.10 S/CO for specimens at 0.80 S/CO (high negative panel).

Within-Laboratory Precision

A study was performed based on guidance from the National Committee for Clinical Laboratory Standards (NCCLS) document EP5-A2.[16] Testing was conducted at Abbott Laboratories using 3 lots of ARCHITECT HBsAg Qualitative reagents, calibrators, and controls, and 3 instruments. Two controls and 3 panels were assayed in a minimum of 2 replicates at 2 separate times per day for 20 different days. Each reagent lot used a single calibration throughout the study. The total imprecision (within-run, between-run, and between-day) across lots and instrument systems for the ARCHITECT HBsAg Qualitative assay was 2.1 to 3.3 %CV for the positive control and positive panels, and the SD was 0.025 to 0.033 S/CO for the high negative panel.

The results are summarized in the following table.

Instrument	Reagent Lot	Sample	N	Mean S/CO	Within-Run		Within-Laboratory Precision (Total)	
					SD	%CV	SD	%CV
i 2000$_{SR}$ (1)	1	Negative Control	118	0.18	0.012	NA	0.030	NA
		Positive Control	119	3.45	0.059	1.7	0.095	2.7
		High Negative Panel	119	0.77	0.022	2.9	0.033	4.3
		Low Positive Panel	119	1.27	0.028	2.2	0.039	3.1
		Moderate Positive Panel	118	3.63	0.071	1.9	0.093	2.6
	2	Negative Control	120	0.17	0.015	NA	0.030	NA
		Positive Control	119	3.44	0.068	2.0	0.099	2.9
		High Negative Panel	119	0.75	0.023	3.1	0.031	4.2
		Low Positive Panel	119	1.25	0.031	2.4	0.040	3.2
		Moderate Positive Panel	120	3.57	0.074	2.1	0.103	2.9
	3	Negative Control	120	0.15	0.012	NA	0.021	NA
		Positive Control	120	3.31	0.063	1.9	0.084	2.5
		High Negative Panel	120	0.72	0.023	3.2	0.031	4.4
		Low Positive Panel	120	1.20	0.032	2.7	0.039	3.3
		Moderate Positive Panel	120	3.40	0.060	1.8	0.088	2.6
i 2000$_{SR}$ (2)	1	Negative Control	119	0.17	0.012	NA	0.017	NA
		Positive Control	120	3.43	0.063	1.8	0.088	2.5
		High Negative Panel	120	0.75	0.023	3.0	0.025	3.3
		Low Positive Panel	120	1.26	0.026	2.1	0.029	2.3
		Moderate Positive Panel	119	3.61	0.066	1.8	0.082	2.3
	2	Negative Control	120	0.16	0.012	NA	0.016	NA
		Positive Control	120	3.43	0.059	1.7	0.086	2.5
		High Negative Panel	119	0.73	0.024	3.2	0.025	3.4
		Low Positive Panel	119	1.23	0.029	2.4	0.033	2.7
		Moderate Positive Panel	120	3.54	0.070	2.0	0.087	2.5
	3	Negative Control	119	0.15	0.012	NA	0.014	NA
		Positive Control	120	3.38	0.056	1.7	0.072	2.1
		High Negative Panel	120	0.72	0.021	2.9	0.027	3.7
		Low Positive Panel	119	1.22	0.032	2.7	0.038	3.1
		Moderate Positive Panel	120	3.47	0.063	1.8	0.075	2.2
i 2000	3	Negative Control	120	0.17	0.014	NA	0.025	NA
		Positive Control	120	3.28	0.073	2.2	0.077	2.3
		High Negative Panel	120	0.73	0.022	3.1	0.029	3.9
		Low Positive Panel	120	1.21	0.030	2.5	0.035	2.9
		Moderate Positive Panel	120	3.42	0.082	2.4	0.098	2.9

System Reproducibility

A 5-day precision study was performed for the ARCHITECT HBsAg Qualitative assay based on guidance from the Clinical and Laboratory Standards Institute (CLSI) document EP15-A2[17] and NCCLS document EP5-A2.[16] Testing was conducted at 3 clinical sites using 3 lots each of ARCHITECT HBsAg Qualitative reagents, calibrators, and controls and one ARCHITECT *i* 2000 or ARCHITECT *i* 2000_{SR} per site. Two controls and 3 panels were assayed in replicates of 4 at 2 separate times of day for 5 days.

The results are summarized in the following table.

Sample	N	Grand Mean	Within-Run		Within-Day		Within-Laboratory Precision (Total)		Precision with Additional Component of Between-Site		Precision with Additional Component of Between-Lot		Precision with Additional Components of Site and Lot (Overall)	
		S/CO	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Control	360	0.17	0.028	NA	0.031	NA	0.031	NA	0.034	NA	0.034	NA	0.035	NA
Positive Control	360	3.45	0.066	1.9	0.070	2.0	0.073	2.1	0.087	2.5	0.137	4.0	0.137	4.0
High Negative Panel	360	0.77	0.037	4.8	0.061	7.9	0.061	7.9	0.063	8.3	0.061	7.9	0.063	8.3
Low Positive Panel	360	1.28	0.066	5.1	0.066	5.1	0.066	5.1	0.069	5.4	0.075	5.9	0.075	5.9
Moderate Positive Panel	360	3.64	0.134	3.7	0.138	3.8	0.138	3.8	0.153	4.2	0.188	5.2	0.188	5.2

Clinical Performance

A prospective multi-center study was conducted to evaluate the ability of the ARCHITECT HBsAg Qualitative assay to detect HBsAg in a group of individuals that would normally be tested in a clinical situation. Of the 2800 specimens tested in the clinical study, 1279 specimens were obtained from individuals with increased risk of HBV infection due to lifestyle, behavior, occupation, or a known exposure event and 675 specimens were obtained from individuals exhibiting signs and symptoms of hepatitis infection.

Specimens (n=1954) from these populations consisted of the following race/ethnic groups:

- 970 (49.64%) Caucasian
- 598 (30.60%) African American
- 311 (15.92%) Hispanic
- 40 (2.05%) Asian
- 5 (0.26%) American Indian/Alaska Native
- 30 (1.54%) Other

Specimens (n=1954) from these specimen populations were obtained from the following collection locations:

- 359 (18.37%) Galveston, TX
- 200 (10.24%) Dallas, TX
- 179 (9.16%) Miami, FL
- 496 (25.38%) St. Petersburg, FL
- 284 (14.53%) Chicago, IL
- 137 (7.01%) Denver, CO
- 33 (1.69%) High Point, NC
- 35 (1.79%) Colton, CA
- 111 (5.68%) Plymouth, MA
- 100 (5.12%) Trinity, FL
- 20 (1.02%) Franklin, TN

Of the 1954 specimens from the increased risk and signs and symptoms populations, 893 (45.70%) were female and 1061(54.30%) were male. Age was not reported for two specimens. Of the remaining 1952 specimens, the mean age was 42 years (age range: 17 to 82 years).

The ARCHITECT HBsAg Qualitative assay was further evaluated by testing a total of 126 pre-selected specimens from acute and chronic HBV infections, which included 8 specimens from subjects with clinically diagnosed acute HBV infection, 29 specimens classified as acute based on four-marker HBV reference testing, 67 specimens from subjects with clinically diagnosed chronic HBV infection defined by the presence of HBsAg for ≥ 6 months, and 22 specimens classified as chronic based on four-marker HBV reference testing.

Each specimen was tested using a comparator HBsAg assay and three HBV reference assays, each detecting a unique serological marker (anti-HBc IgM, total anti-HBc, and anti-HBs). The HBV classification was determined for each specimen based on the reactivity patterns of the four HBV serological marker results. The comparator and reference assays were from a single manufacturer, and testing was performed following manufacturer's instructions.

Each specimen was also tested at one of three clinical sites located in Hershey, PA; Fort Lauderdale, FL; and Aurora, CO, using the ARCHITECT HBsAg Qualitative assay.

Results by Specimen Classification

Following testing with the comparator HBsAg assay and three reference HBV assays, the 1954 specimens from the increased risk and signs and symptoms population plus 126 specimens from individuals with acute or chronic HBV infection were assigned an HBV classification according to the following table. There were 13 unique reference marker patterns observed in the ARCHITECT HBsAg Qualitative clinical study.

Number of Specimens	HBV Reference Markers				HBV Classification
	HBsAg [a]	Anti-HBc IgM	Total Anti-HBc	Anti-HBs	
20	+	–	+	–	Acute
2	+	I	+	–	Acute
26	+	+	+	–	Acute
154	+	–	+	–	Chronic
6	+	–	+	+	Chronic
2	+	–	–	+	Chronic
5	+	+	+	+	Late Acute, Recovering
9	–	+	+	+	Recovering Acute
3	–	+	+	–	Recovering Acute, Undetectable HBsAg
118	–	–	+	–	Distantly Immune, Anti-HBs Not Detected
225	–	–	+	+	Immune Due to Natural Infection
414	–	–	–	+	Immune Due to HBV Vaccination
1096	–	–	–	–	Susceptible
2080					Total

+ = Positive/Reactive, – = Negative/Nonreactive, I = Indeterminate
[a] For HBsAg: + = Repeatedly reactive and confirmed by neutralization when required; – = Reference HBsAg test negative or not confirmed by neutralization.

Comparison of Results

The following table compares the ARCHITECT HBsAg Qualitative assay results with the comparator HBsAg assay final interpretation for each of the HBV classifications for the increased risk and signs and symptoms populations (n = 1954) and the acute or chronic HBV infection populations (n=126). The data are summarized in the following table.

HBV Classification	Comparator HBsAg Final Interpretation			
	Confirmed Positive [a]		Negative/Not Confirmed	
	ARCHITECT HBsAg Qualitative Result		ARCHITECT HBsAg Qualitative Result	
	Reactive	Nonreactive	Reactive	Nonreactive
	N	N	N	N
Acute	47	1	0	0
Chronic	160	2	0	0
Late Acute, Recovering	5	0	0	0
Recovering Acute	0	0	0	9
Recovering Acute, Undetectable HBsAg	0	0	0	3
Distantly Immune, Anti-HBs Not Detected	0	0	3	115
Immune Due to Natural Infection	0	0	5	220
Immune Due to HBV Vaccination	0	0	0	414
Susceptible	0	0	6	1090
Total	212	3 [b]	14 [c]	1851

[a] The comparator HBsAg final positive interpretation includes retesting and confirmatory testing according to the comparator package inserts.
[b] All 3 specimens were positive for an additional marker (anti HBc or anti-HBs) or had DNA present (assay sensitivity of 169 copies/mL).
[c] Of these 14 specimens, 1 specimen was not confirmed on the ARCHITECT HBsAg Qualitative Confirmatory assay, 10 specimens were positive for an additional marker (anti-HBc, anti HBs, or anti-HBe) or had DNA present, and 3 specimens had no additional markers or DNA present.

Percent Agreement

The percent agreement between the ARCHITECT HBsAg Qualitative assay results and the comparator HBsAg assay final interpretation for the increased risk and signs and symptoms populations by HBV classification (n=1954) is summarized in the table below.

HBV Classification	Positive Percent Agreement	95% Confidence Interval	Negative Percent Agreement	95% Confidence Interval
Acute	100.00 (7/7)	(59.04, 100.00)	NA	NA
Chronic	97.65 (83/85)	(91.76, 99.71)	NA	NA
Recovering Acute	NA	NA	100.00 (9/9)	(66.37, 100.00)
Recovering Acute, Undetectable HBsAg	NA	NA	100.00 (3/3)	(29.24, 100.00)
Distantly Immune, Anti-HBs Not Detected	NA	NA	97.44 (114/117)	(92.69, 99.47)
Immune Due to Natural Infection	NA	NA	98.21 (219/223)	(95.47, 99.51)
Immune Due to HBV Vaccination	NA	NA	100.00 (414/414)	(99.11, 100.00)
Susceptible	NA	NA	99.45 (1090/1096)	(98.81, 99.80)
Total	**97.83 (90/92)**	**(92.37, 99.74)**	**99.30 (1849/1862)**	**(98.81, 99.63)**

Percent Agreement for Individuals With Acute or Chronic HBV Infection

The percent agreement between the ARCHITECT HBsAg Qualitative assay results and the comparator HBsAg assay final interpretation for the pre-selected specimens from individuals with acute and chronic HBV infection (n=126) are presented in the table below.

Specimen Category	Positive Percent Agreement	95% Confidence Interval	Negative Percent Agreement	95% Confidence Interval
Individuals with Acute HBV Infection	97.30 (36/37)	(85.84, 99.93)	NA	NA
Individuals with Chronic HBV Infection	100.00 (86/86)	(95.80, 100.00)	66.67 (2/3)	(9.43, 99.16)

Increased Risk Population Testing

In addition to the 1279 specimens from individuals at increased risk tested at 3 clinical sites, 498 specimens from hemodialysis patients were tested at Abbott Laboratories. The following table compares the ARCHITECT HBsAg Qualitative results and comparator HBsAg assay final interpretations for each risk factor for this overall increased risk population.

	Comparator HBsAg Assay Final Interpretation				
	Confirmed Positive		Negative/Not Confirmed		
	ARCHITECT HBsAg Qualitative Result		ARCHITECT HBsAg Qualitative Result		
Specimen Category	Reactive (N)	Nonreactive (N)	Reactive (N)	Nonreactive (N)	Total (N)
Multiple Sex Partners	23	1	3	876	903
Injecting Drug User (IDU)	2	0	1	116	119
Men who have Sex with Men (MSM)	1	0	1	9	11
Sexual Contact with HBV	2	0	0	22	24
Household Contact with HBV	6	0	0	43	49
Occupational Exposure Incident	2	0	1	163	166
Hemodialysis Patient	2	0	0	499	501 [a]
Perinatal Exposure to HBV	2	0	0	2	4
Total	**40**	**1**	**6**	**1730**	**1777**

[a] Of these 501 specimens, 3 specimens were tested at clinical sites and 498 specimens were tested at Abbott Laboratories.

Clinical Performance in Pregnant Females

The performance of ARCHITECT HBsAg Qualitative in detecting HBV infection in pregnant females was evaluated by testing serum specimens from pregnant females at low risk or increased risk of HBV infection due to lifestyle, behavior, or known exposure event. Of the 2800 specimens tested in the clinical study, 720 were from a pregnant female population. The specimens were obtained from commercial vendors. The 720 specimens, from pregnant females aged 16 to 45 years, were collected from collection sites in Colton, CA (n=161); Plymouth, MA (n=7); and Los Angeles, CA (n=552). Testing of these specimens was performed at the clinical sites located in Hershey, PA, Fort Lauderdale, FL, and Aurora, CO.

The demographic profile of the pregnant female population is presented in the table below.

Category	Low Risk N (%)	Increased Risk N (%)	Total N (%)
TOTAL	544 (75.56)	176 (24.44)	720 (100.00)
TRIMESTER			
First	24 (4.41)	6 (3.41)	30 (4.17)
Second	259 (47.61)	68 (38.64)	327 (45.42)
Third	261 (47.98)	102 (57.95)	363 (50.42)
RACE/ETHNIC GROUP			
Caucasian	10 (1.84)	38 (21.59)	48 (6.67)
African American	52 (9.56)	22 (12.50)	74 (10.28)
Hispanic	465 (85.48)	108 (61.36)	573 (79.58)
Asian	15 (2.76)	0 (0.00)	15 (2.08)
American Indian/Alaska Native	0 (0.00)	2 (1.14)	2 (0.28)
Other	2 (0.37)	6 (3.41)	8 (1.11)
AGE RANGE			
16 to 31	320 (58.82)	146 (82.95)	466 (64.72)
32 to 45	224 (41.18)	30 (17.05)	254 (35.28)

Agreement for Pregnant Females by Risk and Trimester

A comparison was performed between the ARCHITECT HBsAg Qualitative assay results and the comparator HBsAg assay results using serum samples obtained from a total of 720 pregnant females at low risk or increased risk for HBV infection. Data were analyzed by risk and by trimester.

The data are summarized in the tables below.

ARCHITECT and Comparator HBsAg Results by Trimester for Low Risk Pregnant Females

	First Trimester			Second Trimester			Third Trimester		
	Comparator HBsAg Final Interpretation			Comparator HBsAg Final Interpretation			Comparator HBsAg Final Interpretation		
ARCHITECT HBsAg Qualitative Result	Confirmed Positive	Negative/Not Confirmed	Total	Confirmed Positive	Negative/Not Confirmed	Total	Confirmed Positive	Negative/Not Confirmed	Total
Reactive	0	0	0	0	0	0	0	0	0
Nonreactive	0	24	24	0	259	259	0	261	261
Total	0	24	24	0	259	259	0	261	261

ARCHITECT and Comparator HBsAg Results by Trimester for Increased Risk Pregnant Females

	First Trimester			Second Trimester			Third Trimester		
	Comparator HBsAg Final Interpretation			Comparator HBsAg Final Interpretation			Comparator HBsAg Final Interpretation		
ARCHITECT HBsAg Qualitative Result	Confirmed Positive	Negative/Not Confirmed	Total	Confirmed Positive	Negative/Not Confirmed	Total	Confirmed Positive	Negative/Not Confirmed	Total
Reactive	0	0	0	0	0	0	1	0	1
Nonreactive	0	6	6	0	68	68	0	101	101
Total	0	6	6	0	68	68	1	101	102

Overall Summary and Percent Agreement for Pregnant Females

The percent agreement between the ARCHITECT HBsAg Qualitative assay results and the comparator HBsAg assay results for the pregnant female population are summarized in the table below.

Subjects	Positive Percent Agreement	95% Confidence Interval	Negative Percent Agreement	95% Confidence Interval
Pregnant Females	100.00% (1/1)	(2.50%, 100.00%)	100.00% (719/719)	(99.49%, 100.00%)

A total of 3172 specimens from a diagnostic population (increased risk for HBV infection, signs and symptoms of hepatitis infection, and pregnant females) were tested using the ARCHITECT HBsAg Qualitative assay. The repeatedly reactive specimens were confirmed using the ARCHITECT HBsAg Qualitative Confirmatory assay. There were 122/3172 (3.85%) initially reactive results and 106/3172 (3.34%) repeatedly reactive results. Of the repeatedly reactive results, 104/106 (98.11%) results were confirmed.

Clinical Performance in a Pediatric Population

Of the 2800 specimens in the clinical study, 142 specimens were from a pediatric population aged 17 to 21. In addition, 68 specimens from pediatric individuals aged 4 to 18 who were at increased risk of HBV infection were tested at Abbott Laboratories. For all 210 specimens, the negative percent agreement was 99.51% (203/204) with a 95% confidence interval of 97.30% to 99.99% and the positive percent agreement was 83.33% (5/6) with a 95% confidence interval of 35.88% to 99.58% for the ARCHITECT HBsAg Qualitative result versus the comparator HBsAg final interpretation.

The ARCHITECT HBsAg Qualitative results are summarized by age and gender in the following table.

Age Range (Years)	Gender	ARCHITECT HBsAg Qualitative Result		Total
		Reactive N (%)	Nonreactive N (%)	
>4 to 12	Female	1 (5.26)	18 (94.74)	19
	Male	0 (0.00)	22 (100.00)	22
>12 to 18	Female	0 (0.00)	23 (100.00)	23
	Male	0 (0.00)	7 (100.00)	7
>18 to 21	Female	2 (1.75)	112 (98.25)	114
	Male	3 (12.00)	22 (88.00)	25
Total		6 (2.86)	204 (97.14)	210

Neonate Serum

A study was conducted to evaluate whether neonate samples may be tested with the ARCHITECT HBsAg Qualitative assay. Cord blood serum was used as a surrogate for neonate serum. Twenty-three matched cord blood and maternal serum samples were spiked with HBsAg positive stock to yield a high negative sample (target S/CO 0.80) and a low positive sample (target S/CO 1.20).

The distribution of the percent differences per analyte level is listed in the following table.

Analyte Level	N	Distribution of Percent Differences			
		< 10%	≥ 10% to < 20%	≥ 20% to < 30%	≥ 30%
0.80 S/CO	23	91.3% (21/23)	8.7% (2/23)	0.0% (0/23)	0.0% (0/23)
1.20 S/CO	23	91.3% (21/23)	8.7% (2/23)	0.0% (0/23)	0.0% (0/23)

90

Analytical Sensitivity (Detectable Concentration of HBsAg at the Cutoff)

The ARCHITECT HBsAg Qualitative assay is designed to have an analytical sensitivity value of less than or equal to 0.20 ng/mL (0.036 IU/mL).

Analytical sensitivity was evaluated using serial dilutions of the WHO 2nd International HBsAg Standard (subtype *adw2*, genotype A, NIBSC Code 00/588). The dilutions ranged from 0.01 to 0.50 IU/mL. Recalcified negative human plasma/serum was used as the diluent and also represented the 0 IU/mL sample. The dilutions were tested across 3 reagent lots on 3 ARCHITECT instruments (2 *i* 2000_{SR} and 1 *i* 2000). The HBsAg level at the assay's cutoff was estimated from a linear regression analysis. The analytical sensitivity for ARCHITECT HBsAg Qualitative ranged from 0.017 to 0.022 IU/mL across the instruments.

In the analytical sensitivity study, the observed limit of detection (LoD), calculated per NCCLS document EP17-A,[18] was less than or equal to 0.002 IU/mL across the instruments.

Analytical Specificity

The ARCHITECT HBsAg Qualitative assay was evaluated for potential cross-reactivity for specimens from individuals with medical conditions unrelated to HBV infection. A total of 301 specimens from 28 different categories were tested. Two hundred ninety-eight (298) specimens were nonreactive and 3 specimens were reactive by the ARCHITECT HBsAg Qualitative and comparator HBsAg assays. All 3 reactive specimens were confirmed positive for HBsAg by the ARCHITECT HBsAg Qualitative Confirmatory and comparator HBsAg confirmatory assays.

The data are summarized by final interpretation in the following table.

		Comparator HBsAg Assay			
		Negative/Not Confirmed		Positive [a]	
		ARCHITECT HBsAg Qualitative		ARCHITECT HBsAg Qualitative	
Category	N	Nonreactive	Reactive	Nonreactive	Reactive
Anti-nuclear antibodies (ANA)	10	10	0	0	0
Auto-immune hepatitis	10	10	0	0	0
C. trachomatis	7	7	0	0	0
Cytomegalovirus (CMV)	10	10	0	0	0
Epstein-Barr virus (EBV)	10	10	0	0	0
Fatty liver disease	10	10	0	0	0
Hemodialysis patient	10	10	0	0	0
Hepatitis A virus (HAV)	10	10	0	0	0
Hepatitis C virus (HCV)	10	10	0	0	0
Hepatocellular carcinoma	10	10	0	0	0
Herpes simplex virus (HSV)	10	10	0	0	0
HIV-1	10	10	0	0	0
HIV-2	17	14	0	0	3
Human anti-mouse antibodies (HAMA) positive	15	15	0	0	0
Human T-lymphotropic virus (HTLV-1/2)	9	9	0	0	0
IgG monoclonal gammopathy	10	10	0	0	0
IgM monoclonal gammopathy	10	10	0	0	0
Influenza vaccine recipients	10	10	0	0	0
Multiparous pregnancies	10	10	0	0	0
Multiple myeloma	10	10	0	0	0
Multiple transfusion recipients	10	10	0	0	0
N. gonorrhea	9	9	0	0	0
Pregnancy 1st trimester	15	15	0	0	0
Pregnancy 2nd trimester	14	14	0	0	0
Pregnancy 3rd trimester	15	15	0	0	0
Rheumatoid arthritis (RF)	10	10	0	0	0
T. cruzi	10	10	0	0	0
T. pallidum	10	10	0	0	0
Total	**301**	**298**	**0**	**0**	**3**

[a] The comparator HBsAg final positive interpretation includes retesting and confirmatory testing according to the comparator package inserts.

In addition, a minimum of 10 serum samples were supplemented with antigens from hepatitis A virus, cytomegalovirus, Epstein-Barr virus, herpes simplex virus-1, rubella, *Toxoplasma gondii*, and varicella-zoster virus. The viral or parasitic antigens were spiked to 1.0 µg/mL except for the hepatitis A virus, which was spiked to 0.1 µg/mL. The prepared samples were tested in replicates of one. All replicates of the serum samples spiked with viral or parasitic antigens were nonreactive.

Interference

At the concentrations listed below, the ARCHITECT HBsAg Qualitative assay showed interference from unconjugated bilirubin, conjugated bilirubin, protein, hemoglobin, and triglycerides for high negative samples (targeted to an S/CO of 0.80) of ≤ +0.15 S/CO and low positive samples (targeted to an S/CO of 1.20) of ≥ -15%.

Interferent	Interferent Concentration
• Unconjugated bilirubin	≤ 20 mg/dL
• Conjugated bilirubin	≤ 20 mg/dL
• Triglycerides	≤ 3000 mg/dL
• Protein	≤ 12 g/dL
• Hemoglobin	≤ 500 mg/dL

Tube Type Matrix Comparison

The following tube types are acceptable for use with the ARCHITECT HBsAg Qualitative assay:

- Serum, including serum separator
- Plasma: dipotassium EDTA, tripotassium EDTA, lithium heparin, lithium heparin separator, and sodium heparin

On average, the tube types listed in the table below showed less than a 15% difference when compared to the control tube type (plastic serum) for low positive samples (S/CO range: 1.00 to 1.40) and less than a 0.15 S/CO difference for high negative samples (S/CO range: 0.60 to 0.99).

The ARCHITECT HBsAg Qualitative assay showed the following distribution of percent differences when compared to the plastic serum tube type.

	Distribution of Differences for High Negative Samples			Distribution of Percent Differences for Low Positive Samples		
Evaluation Tube Type	< 0.10 S/CO	≥ 0.10 S/CO to ≤ 0.20 S/CO	> 0.20 S/CO	< -20%	≥ -20% to ≤ -10%	> -10%
Serum Separator, Plastic	96.4% (27/28)	3.6% (1/28)	0.0% (0/28)	0.0% (0/26)	0.0% (0/26)	100.0% (26/26)
Dipotassium EDTA	96.4% (27/28)	3.6% (1/28)	0.0% (0/28)	0.0% (0/27)	3.7% (1/27)	96.3% (26/27)
Tripotassium EDTA	96.4% (27/28)	3.6% (1/28)	0.0% (0/28)	0.0% (0/27)	0.0% (0/27)	100.0% (27/27)
Lithium Heparin	100.0% (28/28)	0.0% (0/28)	0.0% (0/28)	0.0% (0/27)	0.0% (0/27)	100.0% (27/27)
Sodium Heparin	96.4% (27/28)	3.6% (1/28)	0.0% (0/28)	0.0% (0/27)	3.7% (1/27)	96.3% (26/27)
Lithium Heparin Plasma Separator	100.0% (28/28)	0.0% (0/28)	0.0% (0/28)	0.0% (0/27)	0.0% (0/27)	100.0% (27/27)

Seroconversion Sensitivity

The ARCHITECT HBsAg Qualitative assay is designed to have a seroconversion sensitivity that is better than or equivalent to the seroconversion sensitivity of the comparator HBsAg assay.

To determine the seroconversion sensitivity, 38 HBV seroconversion panels obtained from commercial vendors were tested using the ARCHITECT HBsAg Qualitative and ARCHITECT HBsAg Qualitative Confirmatory assays. HBsAg was first detected by the ARCHITECT HBsAg Qualitative assay and confirmed by the ARCHITECT HBsAg Qualitative Confirmatory assay 2 to 15 days earlier than it was first detected by the comparator HBsAg assay in 17 seroconversion panel sets and coincident with the first day detected by the comparator HBsAg assay in 21 seroconversion panel sets.

The data are summarized in the following table.

	Days to HBsAg Reactive Result from Initial Draw Date		Difference in Days to HBsAg Reactive Result (Comparator – ARCHITECT)		Days to HBsAg Reactive Result from Initial Draw Date		Difference in Days to HBsAg Reactive Result (Comparator – ARCHITECT)
Panel ID	Comparator HBsAg Assay	ARCHITECT HBsAg Qualitative		Panel ID	Comparator HBsAg Assay	ARCHITECT HBsAg Qualitative	
PHM909	9	9	0	6272	101	94	7
PHM917	43	36	7	6273	25	25	0
PHM925	8	4	4	6274	4	0	4
PHM926	13	9	4	6275	22	7	15
PHM927	4	4	0	11000	26	21	5
PHM928	9	7	2	11001	44	44	0
PHM929	18	14	4	11002	9	7	2
PHM930	3	3	0	11003	142	142	0
PHM933	7	7	0	11005	142	142	0
PHM934	0	0	0	11006	51	42	9
PHM935B	128	128	0	11007	43	34	9
PHM935A(M2)	21	21	0	11008	72	69	3
26982/14399	3	3	0	11009	86	79	7
13867/3482	19	19	0	11011	110	103	7
1807/3463	11	11	0	11012	18	18	0
26022/14518	12	12	0	11013	252	247	5
0994/3457	11	4	7	11014	51	51	0
43527/3453	13	13	0	11016	27	27	0
6271	7	7	0	11017	42	42	0

52

HBsAg Mutant Detection

The ARCHITECT HBsAg Qualitative assay is designed to have the ability to better detect (as reactive) the HBsAg mutant Thr-123-Ala and to have the equivalent or better ability to detect (as reactive) other HBsAg mutants (including Gly-145-Arg) when compared to the comparator HBsAg assay.

The hepatitis B virus, unlike other DNA viruses, replicates through reverse transcription. The reverse transcription process lacks proofreading capability; therefore, HBV is subject to a mutation rate 10 times higher than the mutation rate of other DNA viruses.[19] Some of these mutations may cause changes in the antigenic structure of HBsAg, resulting in epitopes that are no longer recognized by anti-HBs. HBsAg mutants have been reported in a wide range of patient populations, including blood donors, vaccine recipients, renal dialysis patients, orthotopic liver transplant recipients, infants born to HBsAg-positive mothers, and patients undergoing nucleoside analog treatment for HBV.[19-26] HBsAg mutations may result in a less favorable outcome in some patients[19,20,22] and false negative results in some HBsAg assays.[19-21]

A panel of 9 HBsAg recombinant proteins containing defined mutations between amino acid positions 122 and 145 were prepared in a fetal calf serum-containing tissue culture media as described by Coleman, Chen, and Mushahwar[27] except for the Thr-123-Ala mutation, which was expressed in serum-free tissue culture media. Each mutant was diluted with recalcified negative human plasma to an S/CO of 2.0 ± 0.5 and tested with the ARCHITECT HBsAg Qualitative assay and with a comparator HBsAg assay.

The data are summarized in the following table.

	Final Results	
Mutant	ARCHITECT HBsAg Qualitative	Comparator HBsAg
Gln-129-His	Repeatedly Reactive	Repeatedly Reactive
Met-133-Leu	Repeatedly Reactive	Repeatedly Reactive
Asp-144-Ala	Repeatedly Reactive	Nonreactive
Gly-145-Arg	Repeatedly Reactive	Repeatedly Reactive
Thr-123-Ala	Repeatedly Reactive	Nonreactive
P142L+G145R	Repeatedly Reactive	Repeatedly Reactive
P142S+G145R	Repeatedly Reactive	Repeatedly Reactive
122NT	Repeatedly Reactive	Repeatedly Reactive
122RA	Repeatedly Reactive	Repeatedly Reactive

HBV Genotype Detection

The ARCHITECT HBsAg Qualitative assay is designed to detect HBV genotypes A through F and H. A study was performed to evaluate the ability of the ARCHITECT HBsAg Qualitative assay to detect HBV genotypes A through H. A total of 18 panel members (3 panel members each of A, B, C, D, and E; 2 panel members each of F and G, and 1 panel member of H) were tested using the ARCHITECT HBsAg Qualitative and ARCHITECT HBsAg Qualitative Confirmatory assays. All genotypes were reactive by the ARCHITECT HBsAg Qualitative assay and confirmed positive by the ARCHITECT HBsAg Qualitative Confirmatory assay.

BIBLIOGRAPHY

1. Neurath AR, Kent SB, Strick N, *et al.* Identification and chemical synthesis of a host cell receptor binding site on hepatitis B virus. *Cell* 1986;46:429-36.
2. Szmuness W, Stevens CE, Harley EJ, *et al.* Hepatitis B vaccine-demonstration of efficacy in a controlled clinical trial in a high-risk population in the United States. *N Engl J Med* 1980;303:833-41.
3. Krugman S, Giles JP. Viral hepatitis, type B (MS-2-Strain)- further observations on natural history and prevention. *N Engl J Med* 1973;288:755-60.
4. Krugman S, Overby LR, Mushahwar IK, *et al.* Viral hepatitis, type B-studies on natural history and prevention re-examined. *N Engl J Med* 1979;300:101-6.
5. Perrillo RP, Aach RD. The clinical course and chronic sequelae of hepatitis B virus infection. *Seminars in Liver Disease* 1981;1:15-25.
6. CDC. A comprehensive immunization strategy to eliminate transmission of Hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP) Part 1: Immunization of Infants, Children, and Adolescents. *MMWR* 2005;54(RR-16):1-23.
7. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Bloodborne pathogens.
8. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; December 2009.
9. World Health Organization. *Laboratory Biosafety Manual*. 3rd ed. Geneva: World Health Organization; 2004.
10. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline—Third Edition*. CLSI Document M29-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
11. Centers for Medicare and Medicaid Services, Department of Health and Human Services. 42 CFR Part 493.1282. Standard: Corrective actions. Paragraph (b)(2).
12. Clinical and Laboratory Standards Institute. *Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline-Third Edition*. CLSI Document C24-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2006.
13. Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. *Clin Chem* 1988;34(1):27-33.
14. Primus FJ, Kelley EA, Hansen HJ, *et al.* "Sandwich"-type immunoassay of carcinoembryonic antigen in patients receiving murine monoclonal antibodies for diagnosis and therapy. *Clin Chem* 1988;34(2):261-4.
15. Schroff RW, Foon KA, Beatty SM, *et al.* Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy. *Cancer Res* 1985;45:879-85.
16. National Committee for Clinical Laboratory Standards (NCCLS). *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline–Second Edition*. NCCLS Document EP5-A2. Wayne, PA: NCCLS; 2004.
17. Clinical and Laboratory Standards Institute. *User Verification of Performance for Precision and Trueness; Approved Guideline - Second Edition*. CLSI Document EP15-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
18. National Committee for Clinical Laboratory Standards (NCCLS). *Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline*. NCCLS Document EP17-A. Wayne, PA: NCCLS; 2004.
19. Hunt CM, McGill JM, Allen MI, *et al.* Clinical relevance of hepatitis B viral mutations: *Hepatology* 2000;31(5):1037-44.
20. Locarnini SA. Hepatitis B virus surface antigen and polymerase gene variants: potential virological and clinical significance: *Hepatology* 1998;27(1):294–7.
21. Zuckerman AJ. Effect of hepatitis B virus mutants on efficacy of vaccination. *Lancet* 2000;355:1382-4.
22. Carman WF, Trautwein C, Van Deursen FJ, *et al.* Hepatitis B virus envelope variation after transplantation with and without hepatitis B immune globulin prophylaxis. *Hepatology* 1996;24(3):489-93.
23. Grethe S, Monazahian M, Böhme I, *et al.* Characterization of unusual escape variants of hepatitis B virus isolated from a hepatitis B surface antigen-negative subject. *J Virology* 1998;72(9):7692-6.
24. Nainan OV, Stevens CE, Taylor PE, *et al.* Hepatitis B virus (HBV) antibody resistant mutants among mothers and infants with chronic HBV infection. In: Rizzetto M, Purcell RH, Gerin JL, *et al.*, eds. *Viral Hepatitis and Liver Disease*. Minerva Medica: Torino;1997:132-4.
25. Jongerius JM, Wester M, Cuypers HTM, *et al.* New hepatitis B virus mutant form in a blood donor that is undetectable in several hepatitis B surface antigen screening assays. *Transfusion* 1998;38:56-9.
26. Bock CT, Tillmann HL, Torresi J, *et al.* Selection of hepatitis B virus polymerase mutants with enhanced replication by lamivudine treatment after liver transplantation. *Gastroenterology* 2002;122:264-73.
27. Coleman PF, Chen Y-CJ, Mushahwar IK. Immunoassay detection of hepatitis B surface antigen mutants. *J Med Vir* 1999;59:19-24.

The following U.S. Patents are relevant to the ARCHITECT System or its components. There are other such patents and patent applications in the United States and worldwide.

5 468 646	5 543 524	5 545 739
5 565 570	5 669 819	5 783 699

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